

Table S2. Characterization of PGHs identified in the screening by time-kill (TKA), turbidity reduction (TRA), minimum inhibitory concentration (MIC) and stability (SA) assays. Results of each assay were normalized to the highest scorer. The final score was calculated based on a weighted sum: 4×TKA + 2×MIC + 2×TRA + 2×SA. Enzymes were grouped into three categories based on their cleavage specificity: (i) Gly - Gly endopeptidases, (ii) Gly - D-Ala endopeptidases, (iii) double- and triple-acting enzymes. The highest-scoring enzyme from each category (shown in bold) was selected for further experiments. ND – not determined.

| | | TKA | | | | TRA | | MIC | | SA | | Final score | | |
|----------------------------|--------|----------------------------------|--------|--------|---------|---|--------|----------|--------|------------------------|--------|---------------------------------|------|------|
| | | log reduction | | | | specific activity [ΔODmin ⁻¹ μM ⁻¹] | rating | MIC [nM] | rating | remaining activity [%] | rating | 4×TKA + 2×MIC + 2×TRA + 2×SA | | |
| | | 10 min | 30 min | 60 min | average | | | | | | | | | |
| Gly - Gly endopeptidases | PGH 1 | Lysostaphin | 5.86 | 5.87 | 5.99 | 5.91 | 10.0 | 1.10 | 3.6 | 15.625 | 10.0 | 44.22 | 4.6 | 76.4 |
| | PGH 2 | M23LST(L)_SH3b2638 | 5.86 | 5.87 | 5.99 | 5.91 | 10.0 | 1.35 | 4.4 | 3.125 | 8.9 | 57.37 | 6.0 | 78.6 |
| | PGH 3 | M23LST_SH3b2638 | 4.04 | 4.87 | 5.46 | 4.79 | 8.1 | 0.92 | 3.0 | 15.625 | 6.4 | 53.20 | 5.5 | 62.4 |
| | PGH 4 | (M23LST)2_SH3b2638 | 1.69 | 3.87 | 4.35 | 3.30 | 5.6 | 0.97 | 3.1 | 31.25 | 5.4 | 96.20 | 10.0 | 65.8 |
| | PGH 5 | (M23LST)2_SH3bALE1 | 3.73 | 5.35 | 5.99 | 5.02 | 8.5 | 1.94 | 6.3 | 15.625 | 6.4 | 55.81 | 5.8 | 64.8 |
| | PGH 6 | H_TEV_(M23LST)2_SH3bLST | 3.04 | 3.31 | 4.42 | 3.59 | 6.1 | 1.06 | 3.5 | 7.8125 | 7.5 | 80.99 | 8.4 | 63.1 |
| | PGH 7 | H_(LST)2 | 0.56 | 2.19 | 3.76 | 2.17 | 3.7 | 0.14 | 0.5 | 15.625 | 6.4 | 72.07 | 7.5 | 43.5 |
| | PGH 8 | H_M23LST_SH3bLST_M23LST | 3.19 | 4.01 | 4.46 | 3.89 | 6.6 | 1.15 | 3.7 | 62.5 | 4.3 | 56.83 | 5.9 | 54.2 |
| Gly - D-Ala endopeptidases | PGH 9 | CHAPTw_SH3b2638 | 3.77 | 4.23 | 4.46 | 4.16 | 7.0 | 0.87 | 2.8 | 15.625 | 6.4 | 6.03 | 0.6 | 47.9 |
| | PGH 10 | CHAP-1_CBD-1 | 3.96 | 5.35 | 5.46 | 4.92 | 8.3 | 0.58 | 1.9 | 250 | 2.1 | 49.35 | 5.1 | 51.7 |
| | PGH 11 | CHAPGH15_SH3bALE1 | 3.55 | 5.87 | 5.99 | 5.14 | 8.7 | 3.07 | 10.0 | 3.125 | 8.9 | 78.46 | 8.2 | 89.0 |
| | PGH 12 | CHAPK_SH3bLST_H | 2.56 | 3.27 | 3.45 | 3.09 | 5.2 | 0.46 | 1.5 | 7.8125 | 7.5 | 71.98 | 7.5 | 53.9 |
| | PGH 13 | CHAP187_SH3bK_H | 4.34 | 5.87 | 5.99 | 5.40 | 9.1 | 2.32 | 7.6 | 62.5 | 4.3 | 2.17 | 0.2 | 60.7 |
| | PGH 14 | (CHAPGH15)2_SH3b2638 | -0.01 | 0.24 | 1.43 | 0.55 | 0.9 | 0.19 | 0.6 | >1000 | 0.0 | 51.30 | 5.3 | 15.7 |
| | PGH 15 | (CHAPGH15)2_SH3bALE1 | 2.19 | 3.57 | 3.46 | 3.07 | 5.2 | 0.14 | 0.5 | 31.25 | 5.4 | 20.36 | 2.1 | 36.7 |
| | PGH 16 | H_Xa_(CHAPTw)2_SH3b2638 | 0.04 | 0.12 | 0.02 | 0.06 | 0.1 | 0.03 | 0.1 | >1000 | 0.0 | ND | 0.0 | 0.6 |
| | PGH 17 | H_Xa_(CHAPTw)2_SH3bLST | 0.10 | 0.15 | 0.26 | 0.17 | 0.3 | 0.07 | 0.2 | >1000 | 0.0 | ND | 0.0 | 1.6 |
| double and triple acting | PGH 18 | CHAPTw_M23LST(L)_SH3b2638 | 3.64 | 5.35 | 5.99 | 4.99 | 8.5 | 1.18 | 3.9 | 15.625 | 6.4 | 38.19 | 4.0 | 62.3 |
| | PGH 19 | M23LST_Ami2638_SH3b2638 | 1.42 | 3.05 | 4.10 | 2.86 | 4.8 | 1.44 | 4.7 | 500 | 1.1 | 43.87 | 4.6 | 40.0 |
| | PGH 20 | CHAPK_AmiK_SH3bLST_H | 4.30 | 5.35 | 5.16 | 4.94 | 8.4 | 0.23 | 0.8 | 15.625 | 6.4 | 18.06 | 1.9 | 51.6 |
| | PGH 21 | CHAPH5_LST_H | 2.27 | 3.27 | 4.46 | 3.33 | 5.6 | 0.26 | 0.8 | 15.625 | 6.4 | 7.49 | 0.8 | 38.7 |
| | PGH 22 | LST_CHAPK_AmiK_H | 1.51 | 2.78 | 4.35 | 2.88 | 4.9 | 0.29 | 1.0 | 125 | 3.2 | 67.62 | 7.0 | 41.9 |
| | PGH 23 | CHAPH5_AmiH5_LST_H | 0.10 | 1.93 | 4.21 | 2.08 | 3.5 | 0.36 | 1.2 | 62.5 | 4.3 | 68.44 | 7.1 | 39.2 |
| | PGH 24 | LST_CHAPH5_AmiH5(L)_H | 0.03 | 0.54 | 2.28 | 0.95 | 1.6 | 0.29 | 0.9 | 250 | 2.1 | 79.56 | 8.3 | 29.2 |
| | PGH 25 | CHAP11_Ami11_LST_H | 1.27 | 3.54 | 5.46 | 3.42 | 5.8 | 0.25 | 0.8 | 62.5 | 4.3 | 57.55 | 6.0 | 45.4 |